Using Nanoscale Technology to Help Stroke Victims

Prototype Nanoparticles for Future Magnetically Guided, Targeted Tissue Plasminogen Activator Stroke Therapy

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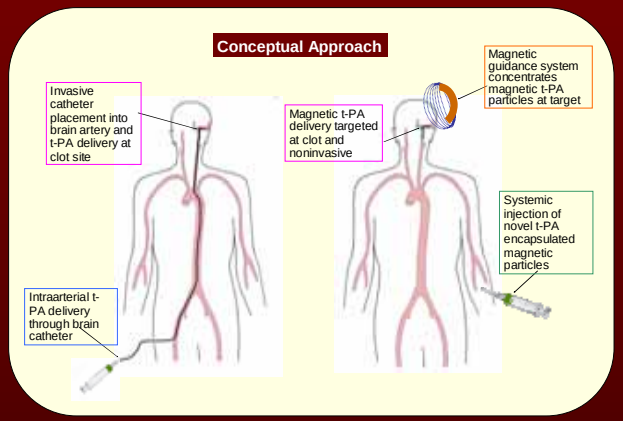
Abstract

A novel strategy for acute stroke treatment based on nanoscale technology could combine the advantages of non-invasive and targeted tPA delivery. In such a system, non-toxic magnetic spheres are loaded with tPA (*tPA-encapsulation*) then intravenously injected and concentrated at the site of the vascular occlusion by an external magnetic field (*targeted lysis*), thereby, potentially improving clot lysis efficiency, reducing side effects, and extending the therapeutic time window. We have identified the first building block of the proposed therapy--the development of tPA-encapsulated magnetic nanocarriers. Biodegradable poly(lactic-co-glycolic) acid microspheres were synthesized to encapsulate dye, protein, and magnetic nanocrystals.



Catheter-Based Brain Interventions: Risky Business

- More than 3 hours after stroke onset, the risk for intravenous (systemic) t-PA injection is too high, excluding most patients from effective stroke therapy.
- Some specialized centers perform intraarterial thrombolysis (catheter-delivered t-PA directly into the blood clot). Major advantages are:
  - Extension of effective-treatment time window to 6 hours
  - Targeted delivery to clot, requiring less total t-PA dose.
- *BUT--catheter-based brain interventions are high-risk procedures, mandating specialized staff around the clock, and are therefore not available to most stroke patients.*

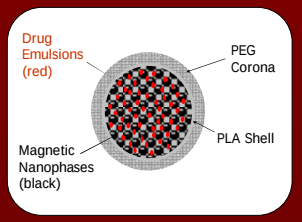
Proposed Drug Delivery System: Minimizes Therapy Risk

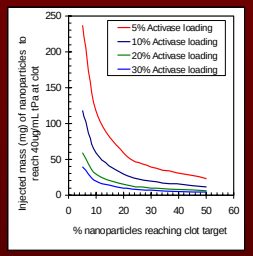
- Would minimize therapy risk and extend the therapeutic window by combining the advantages of intravenous and catheter-based t-PA delivery:
  - Small dose of "designer" magnetic particles containing encapsulated active t-PA injected into the vein (systemic but non-invasive) and magnetically guided and trapped at blood clot site by externally applied magnetic fields.
  - Plasminogen activator release from the particle matrix is spontaneous, possibly by focused ultrasonics.
  - As t-PA encapsulated particles are magnetically guided to and concentrated at the clot site, successful lysis can be achieved with a relatively small dose of t-PA.
- *This delivery concept could lead to decreased systemic side effects and increased time window for successful treatment.*

Nanoparticle Design Goal

To obtain a monodisperse (single size) population of biodegradable spheres composed of poly(lactic acid) derivatives (PLA, i.e., internal-suture polymers) and a homogeneous dispersion of magnetic nanophases (black dots) and drug emulsions (red dots).

The surface layer of covalently bound poly(ethylene glycol) (PEG) "hides" the spheres from opsonization and phagocytosis.





Projections

According to engineering calculations, obtaining a therapeutic level of 4 μg/mL tPa is reasonable. The graph shows the injectable mass of nanoparticles needed to obtain 10 times the therapeutic level (40 μg/mL) at the clot as a function of Activase® loading, and the percent of injected nanoparticles targeted to the clot.

Assumptions: Nanoparticles concentrate in 2.5 cm of a 1.5-μm-diameter artery approaching the clot; 2.2% tPa in Activase; 30% of tPa released spontaneously with 90% activity retained.

Procedure

Oil phase containing biodegradable polymer and drug emulsion is mixed with an external water phase to harden the spheres.

- **Oil phase:** BSA aqueous solution is prepared by dissolving BSA in 100 μL distilled water. PLA-PEG copolymer is dissolved in 2 mL dichloromethane/acetone (1:1) to 5% concentration. BSA solution is emulsified in the PLA-PEG solvent to form a primary emulsion.
- **Water phase:** 4% PVA (Mw 30,000~70,000) in distilled water.
- **Sphere formation and hardening:** Primary emulsion is added to the water phase and stirred for 20 min at 800 rpm and thereafter at 250 rpm overnight. The dichloromethane/acetone evaporates from the solution, leaving a hardened PLA-PEG shell containing the drug emulsion.

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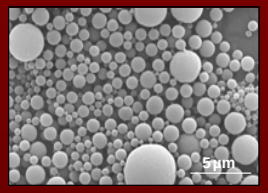
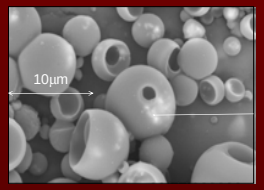
Results

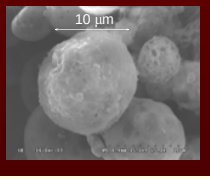
Our results indicate the need to incorporate an oily core composed of FDA-approved fatty acids (oils). This oil would "fill" the inside of the PLA-PEG shell and improve encapsulation of the drug and magnetic phases within the sphere.



A method called solvent evaporation is used to generate the smooth spherical shells (hollow) of poly(lactic-glycolic acid). Inherent with this technique, the resulting spheres are polydisperse, and there is little control over the variance in sphere diameter. Despite a full-parametric study, we are now developing a new technique to replace solvent evaporation.

Poorly sized magnetic nanophases aggregate (white spots) near the surface without the oily core present. Moreover, spheres collapse when we load them with nano-iron. Introducing an oily core should reduce collapsing and promote dispersion of nano-iron.





This micrograph demonstrates the problems associated with encapsulating a hydrophilic drug in an oily capsule. The surface is riddled with holes where the hydrophilic drug globules have penetrated. Such globule distribution would cause a "burst" effect, where the drug dissolves immediately upon injection.

Encapsulation of capric fatty acids (oil) inside the PLA shell appears to form a smooth sphere.

Summary and Conclusions

Our results show that we may need to include an oily core to stabilize the encapsulation of the drug emulsion and magnetic nanophases and create a good prototype particle. We are developing a membrane emulsification technique combined with solvent evaporation to produce a predetermined monodisperse population of particles. This technique prescribes the pumping of the primary emulsion through a filter containing uniform pore sizes, thus controlling the sphere size in the range of 100 nm to several micrometers in diameter.